

A ication No. 09/626,566  
Filed: July 27, 2000  
Group Art Unit: 1651

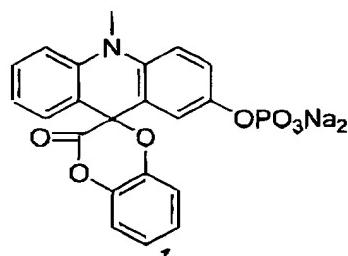
AMENDMENT TO THE CLAIMS

1-7. (Cancelled)

8. (Currently amended) The chemiluminescent substrate of claim 4  
43 wherein said counter ions A are selected from the group  
consisting of  $\text{CH}_3\text{SO}_4^-$ ,  $\text{FSO}_3^-$ ,  $\text{CF}_3\text{SO}_3^-$ ,  $\text{C}_4\text{F}_9\text{SO}_3^-$ ,  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$ , halide,  
 $\text{CF}_3\text{COO}^-$ ,  $\text{CH}_3\text{COO}^-$ , and  $\text{NO}_3^-$ .

9-21. (Cancelled)

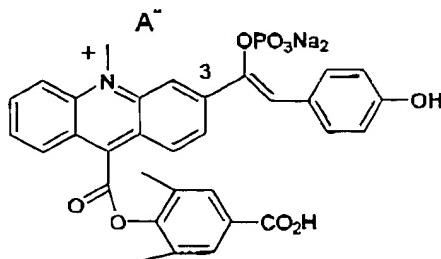
22. (Currently amended) The chemiluminescent substrate of claim  
21 61 having the following structure:



23-24. (Cancelled)

25. (Currently amended) The A chemiluminescent substrate of  
claim 23 having the following structure:

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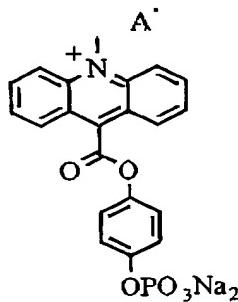


wherein  $\text{A}^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said counter ion  $\text{A}^-$  is selected from the group consisting of  $\text{CH}_3\text{SO}_4^-$ ,  $\text{FSO}_3^-$ ,  $\text{CF}_3\text{SO}_3^-$ ,  $\text{C}_4\text{F}_9\text{SO}_3^-$ ,  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$ , halide,  $\text{CF}_3\text{COO}^-$ ,  $\text{CH}_3\text{COO}^-$ , and  $\text{NO}_3^-$ .

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26-28. (Cancelled)

29. (Currently amended) The A chemiluminescent substrate of claim 26 having the following structure:

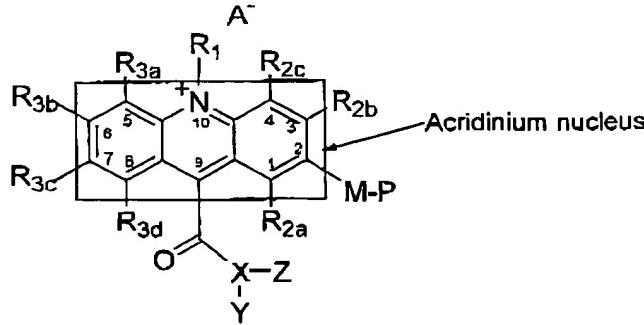


wherein  $\text{A}^+$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said counter ion  $\text{A}^+$  is selected from the group consisting of  $\text{CH}_3\text{SO}_4^-$ ,  $\text{FSO}_3^-$ ,  $\text{CF}_3\text{SO}_3^-$ ,  $\text{C}_4\text{F}_9\text{SO}_3^-$ ,  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3^-$ , halide,  $\text{CF}_3\text{COO}^-$ ,  $\text{CH}_3\text{COO}^-$ , and  $\text{NO}_3^-$ .

30-42. (Cancelled)

43. (Currently amended) The A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure

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wherein

P is  $\text{PO}_3\text{Na}_2$  or a sugar moiety;

M is oxygen;

$R_1$  is selected from the group consisting of methyl, sulfopropyl and sulfobutyl;

$R_{2a}$ ,  $R_{2b}$ ,  $R_{2c}$ ,  $R_{3a}$ ,  $R_{3b}$ ,  $R_{3c}$  and  $R_{3d}$ , are hydrogen;

$A^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said  $A^-$  not being present if said  $R_1$  substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

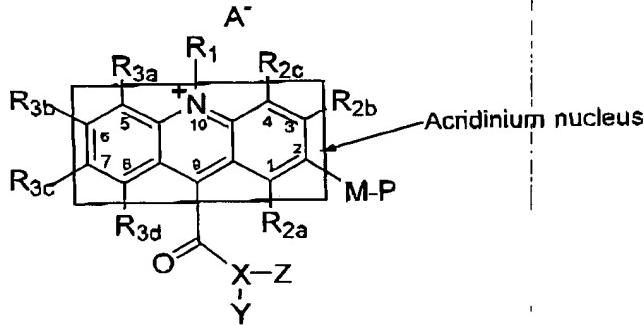
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

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44. (Currently amended) The A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure,



wherein

P is PO<sub>3</sub>Na<sub>2</sub> or a sugar moiety;

M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl, sulfopropyl and sulfobutyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, R<sub>3b</sub>, R<sub>3c</sub> and R<sub>3d</sub>, are hydrogen;

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

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X is O; Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted.

45. (Previously added) The chemiluminescent substrate of claim 43, wherein

P is PO<sub>3</sub>Na<sub>2</sub>;

X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

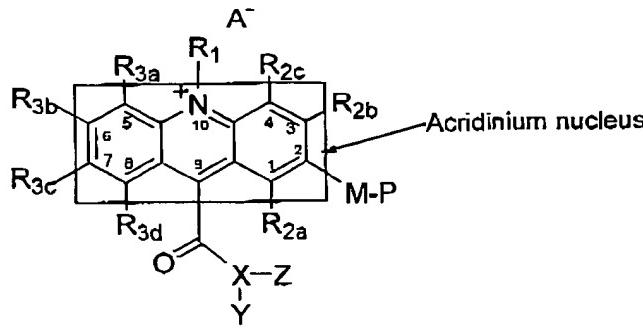
46. (Previously added) The chemiluminescent substrate of claim 43, wherein

P is PO<sub>3</sub>Na<sub>2</sub>;

X is S; Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted.

47. (New) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure

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wherein

P is  $\text{PO}_3\text{Na}_2$  or a sugar moiety;

M is oxygen;

$R_1$  is selected from the group consisting of sulfoalkyl and carboxymethyl;

$R_{2a}$ ,  $R_{2b}$ ,  $R_{2c}$ ,  $R_{3a}$ ,  $R_{3b}$ ,  $R_{3c}$  and  $R_{3d}$ , can be the same or different, selected from the group consisting of hydrogen, methyl, methoxy, halides, and cyano (-CN);

$A^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said  $A^-$  not being present if said  $R_1$  substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

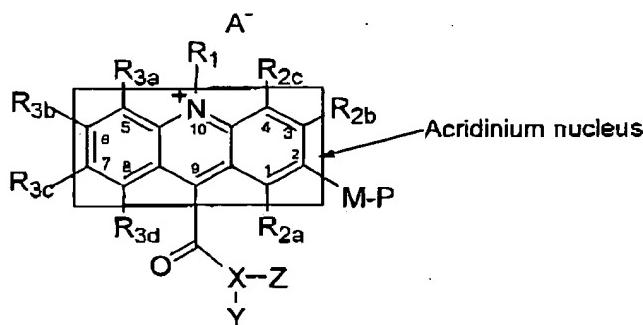
when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-

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benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

48. (New) The chemiluminescent substrate of claim 47 wherein said counter ions A<sup>-</sup> are selected from the group consisting of CH<sub>3</sub>SO<sub>4</sub><sup>-</sup>, FSO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, C<sub>4</sub>F<sub>9</sub>SO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub><sup>-</sup>, halide, CF<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, and NO<sub>3</sub><sup>-</sup>.

49. (New) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

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P is selected from the group consisting of PO<sub>3</sub>H<sub>2</sub>, PO<sub>3</sub>K<sub>2</sub>, PO<sub>3</sub>(NH<sub>4</sub>)<sub>2</sub>, PO<sub>3</sub>Ca, PO<sub>3</sub>Mg and C(=O)R group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, R<sub>3b</sub>, R<sub>3c</sub> and R<sub>3d</sub>, can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, and cyano (-CN);

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

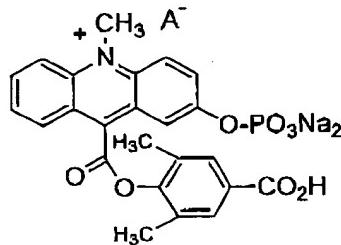
when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl,

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(2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl,; and Z is omitted; and when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

50. (New) The chemiluminescent substrate of claim 49 wherein said counter ions A<sup>-</sup> are selected from the group consisting of CH<sub>3</sub>SO<sub>4</sub><sup>-</sup>, FSO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, C<sub>4</sub>F<sub>9</sub>SO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub><sup>-</sup>, halide, CF<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, and NO<sub>3</sub><sup>-</sup>.

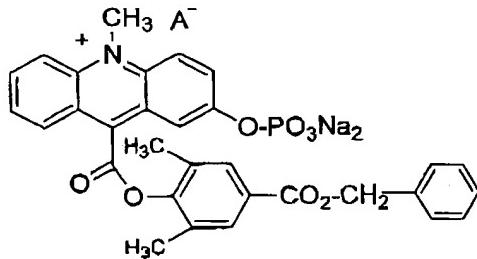
51. (New) The chemiluminescent substrate of Claim 43 having the structure,



wherein A<sup>-</sup> is selected from the group consisting of CH<sub>3</sub>SO<sub>4</sub><sup>-</sup>, FSO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, C<sub>4</sub>F<sub>9</sub>SO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub><sup>-</sup>, halide, CF<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, and NO<sub>3</sub><sup>-</sup>.

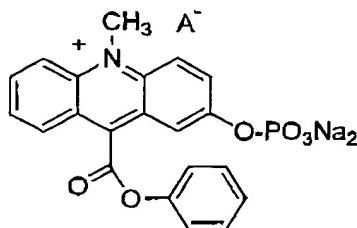
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52. (New) The chemiluminescent substrate of Claim 43 having the structure,



wherein A<sup>-</sup> is selected from the group consisting of CH<sub>3</sub>SO<sub>4</sub><sup>-</sup>, FSO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, C<sub>4</sub>F<sub>9</sub>SO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub><sup>-</sup>, halide, CF<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, and NO<sub>3</sub><sup>-</sup>.

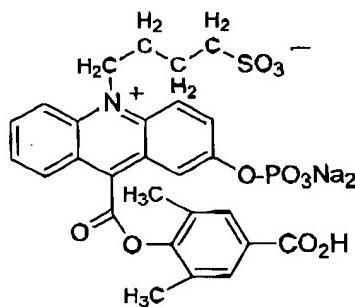
53. (New) The chemiluminescent substrate of Claim 43 having the structure,



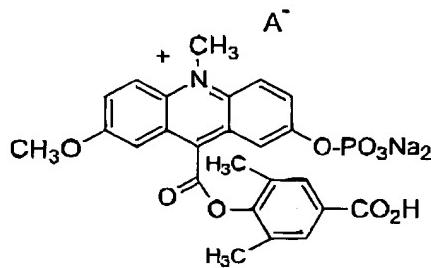
wherein A<sup>-</sup> is selected from the group consisting of CH<sub>3</sub>SO<sub>4</sub><sup>-</sup>, FSO<sub>3</sub><sup>-</sup>, CF<sub>3</sub>SO<sub>3</sub><sup>-</sup>, C<sub>4</sub>F<sub>9</sub>SO<sub>3</sub><sup>-</sup>, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub><sup>-</sup>, halide, CF<sub>3</sub>COO<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, and NO<sub>3</sub><sup>-</sup>.

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54. (New) The chemiluminescent substrate of Claim 43 having the structure



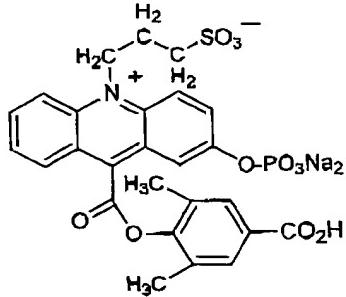
55. (New) The chemiluminescent substrate of Claim 47 having the structure,



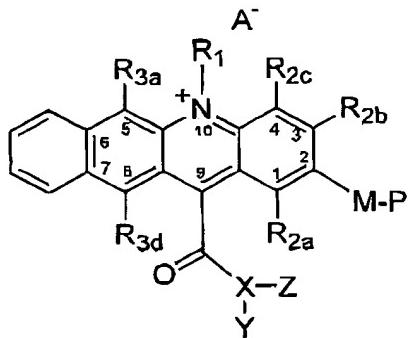
wherein A- is selected from the group consisting of CH3SO4^-, FSO3^-, CF3SO3^-, C4F9SO3^-, CH3C6H4SO3^-, halide, CF3COO^-, CH3COO^-, and NO3^-.

56. (New) The chemiluminescent substrate of Claim 43 having the structure

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57. (New) A chemiluminescent substrate of having the structure



wherein

P is selected from the group consisting of PO3H2, PO3K2, PO3(NH4)2, PO3Ca, PO3Mg, PO3Na2, a sugar moiety and C(=O)R group wherein R is an alkyl group having 1 to 6 carbon atoms;

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M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, and R<sub>3d</sub>, can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano (-CN);

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

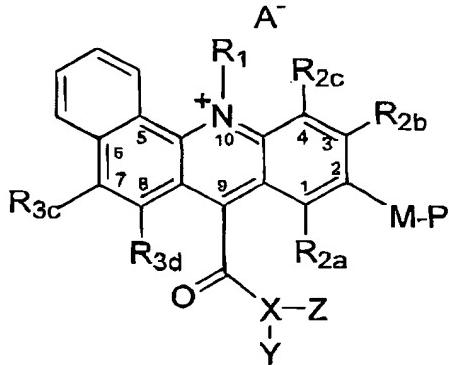
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

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58. (New) A chemiluminescent substrate having the structure



wherein

P is selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,  $\text{PO}_3\text{K}_2$ ,

$\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$ ,  $\text{PO}_3\text{Na}_2$ , a sugar moiety and  $\text{C}(\equiv\text{O})\text{R}$  group

wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

$\text{R}_1$  is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

$\text{R}_{2a}$ ,  $\text{R}_{2b}$ ,  $\text{R}_{2c}$ ,  $\text{R}_{3c}$  and  $\text{R}_{3d}$ , can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, and cyano (-CN);

$\text{A}^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said  $\text{A}^-$  not being

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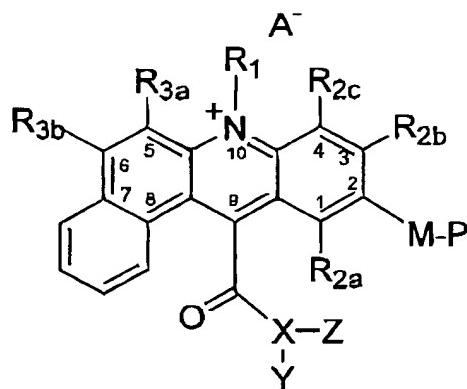
present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

59. (New) A chemiluminescent substrate having the structure



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wherein

P is selected from the group consisting of PO<sub>3</sub>H<sub>2</sub>, PO<sub>3</sub>K<sub>2</sub>, PO<sub>3</sub>(NH<sub>4</sub>)<sub>2</sub>, PO<sub>3</sub>Ca, PO<sub>3</sub>Mg, PO<sub>3</sub>Na<sub>2</sub>, a sugar moiety and C(=O)R group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R<sub>1</sub> is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, and R<sub>3b</sub> can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano (-CN), ;

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

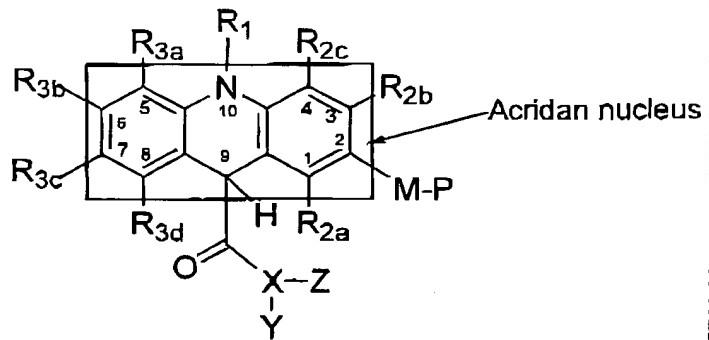
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-

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benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl,  
 (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-  
 carboxyl)phenyl,; and Z is omitted; and  
 when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

60. (New) A chemiluminescent substrate of a hydrolytic enzyme,  
 said substrate having the structure



wherein

P is selected from the group consisting of PO<sub>3</sub>H<sub>2</sub>, PO<sub>3</sub>K<sub>2</sub>, PO<sub>3</sub>(NH<sub>4</sub>)<sub>2</sub>, PO<sub>3</sub>Ca, PO<sub>3</sub>Mg, PO<sub>3</sub>Na<sub>2</sub>, a sugar moiety and C(=O)R group  
 wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

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R<sub>1</sub> is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R<sub>2a</sub>, R<sub>2b</sub>, R<sub>2c</sub>, R<sub>3a</sub>, R<sub>3b</sub>, R<sub>3c</sub> and R<sub>3d</sub>, can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano (-CN), ;

A<sup>-</sup> is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A<sup>-</sup> not being present if said R<sub>1</sub> substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

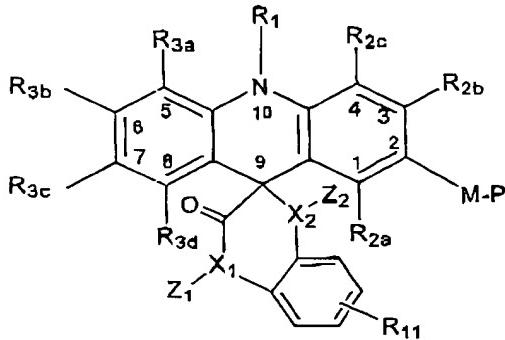
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl;; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

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61. (New) A chemiluminescent substrate of a hydrolytic enzyme,  
 said substrate having the structure



wherein

P is selected from the group consisting of  $\text{PO}_3\text{H}_2$ ,  $\text{PO}_3\text{K}_2$ ,  $\text{PO}_3(\text{NH}_4)_2$ ,  $\text{PO}_3\text{Ca}$ ,  $\text{PO}_3\text{Mg}$ ,  $\text{PO}_3\text{Na}_2$ , a sugar moiety and  $\text{C}(\text{=O})\text{R}$  group  
 wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

$\text{R}_1$  is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

$\text{R}_{2a}$ ,  $\text{R}_{2b}$ ,  $\text{R}_{2c}$ ,  $\text{R}_{3a}$ ,  $\text{R}_{3b}$ ,  $\text{R}_{3c}$  and  $\text{R}_{3d}$ , can be the same or different, selected from a group consisting of hydrogen, methyl, methoxy, halides, cyano ( $-\text{CN}$ );

$\text{A}^-$  is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said  $\text{A}^-$  not being present if said  $\text{R}_1$  substituent contains a strongly ionizable group

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that can form an anion and pair with the quaternary ammonium cationic moiety; and

X<sub>1</sub> and X<sub>2</sub> are the same or different and are selected from the group consisting of O, N or S, such that,

when X<sub>1</sub> and X<sub>2</sub> are O or S, R<sub>11</sub> is selected from the group consisting of hydrogen, -R, substituted or unsubstituted aryl, halides, nitro, sulfonate, sulfate, phosphonate, -CO<sub>2</sub>H, -C(O)OR, cyano (-CN), -SCN, -OR, -SR, -SSR, -C(O)R, -C(O)NHR, ethylene glycol, or polyethylene glycol, where R is as defined above; and Z<sub>1</sub> and Z<sub>2</sub> are omitted; and

when at least one of X<sub>1</sub> and X<sub>2</sub> is N, Z<sub>1</sub> and Z<sub>2</sub> are toluenesulfonyl, and R<sub>11</sub> is carboxypropyl.